U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20090-3630

CHAPTER 11

STRATEGIC DIAGNOSTICS INC. - MYCO✔ DON TEST KIT

11.1 TESTING AREA

The extraction solution and other materials used in the Myco✓ DON test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

11.2 EXTRACTION PROCEDURES

a. Weigh 50 grams of ground sample and place in a clean blender container with a tight fitting lid.

b. Add 250 ml of distilled or deionized water.

c. Blend at high speed for 2 minutes.

d. Allow the extract to stand for 2-3 minutes to allow the sample slurry to settle.

e. Filter a minimum of 15 ml of the extract through a Whatman #1 filter and collect the extract in a clean container that is labeled with the sample ID number.

f. Proceed to the test procedures.

11.3 PREPARATION OF SOLUTIONS

a. To prepare the Wash Solution, transfer the contents of the Wash Concentrate vial to a 500 ml plastic squeeze bottle and add 475 ml of distilled or deionized water.

b. Swirl to mix.

11.4 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

(3) Dispense 100 µl of enzyme conjugate into each well using a pipette.

(4) Using a clean pipette tip for each transfer, dispense 100 µl of each calibrator and sample into the appropriate mixing wells using an adjustable or fixed 100 µl pipette.

Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C 0	C .25	C .5	C 1	C 3	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, C .25 is the 0.25 ppm control, C .5 is the 0.5 ppm control, C 1 is the 1.0 ppm control, and C3 is the 3.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

(5) Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.

(6) Using a multi-channel pipette, transfer 100 µl of each reaction mixture directly into all corresponding clear test wells. Discard the mixing wells into an appropriate waste container.

(7) Let the reaction mixture incubate for exactly 5 minutes. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

(8) At the end of the 5 minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500 ml squeeze bottle containing the wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash for a total of four washes. After the last wash, invert the wells and tap on absorbent paper to remove the residual wash solution. Wipe excess liquid from the bottom of the wells.

(9) Pour the Substrate Solution into a clean reagent reservoir.

(10) Dispense 100 µl of Substrate Solution into each test well using a multi-channel pipette.

(11) Let the Substrate Solution incubate for exactly 5 minutes. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

(12) Pour the Stop Solution into a clean reagent reservoir.

(13) Dispense 100 µl of Stop Solution into each test well using a multi-channel pipette.

(14) Within 20 minutes, read and record the optical density at 650 nm using a Hyperion MicroReader™ 3 Model 4027-002 microwell reader. Make sure that the well bottoms are clean and dry before placing in reader.

b. Reading the Results.

(1) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(2) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(3) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(4) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(5) The reader will read the optical density of the wells and print a report.

(6) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(7) Use the data reduction software provided by SDI to quantify results.

11.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 3.4 ppm. Results exceeding 3.4 ppm are reported as > 3.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 3.4 ppm are certified to the nearest whole ppm.

Test results over 3.4 ppm are certified as exceeding 3 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

11.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 6.0 ppm and the conformance limit value is 3 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 2.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 6.3 ppm (3 x 2.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 2.1 ppm
= 3 x 2.1 ppm = 6.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

11.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

11.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

11.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits.

(1) 48 antibody-coated microtiter wells (4 strips of 12) in foil pouch.

(2) 48 red-marked mixing wells in poly bag.

(3) 5 vials each containing 2 ml of: 0, 0.25, 0.5, 1.0, and 3.0 ppm of DON calibration.

(4) 1 vial containing 8 ml of DON-HRP Enzyme Conjugate.

(5) 1 vial containing 8 ml of Substrate.

(6) 1 vial containing 8 ml of Stop Solution.

(7) 1 vial containing 25 ml of 20X Wash Concentrate.

(8) 4 multi-channel pipette reservoirs.

(9) Data reduction software. (Provided separately)

b. Materials Required but not Provided.

(1) Distilled/deionized water.

(2) 100 ml graduated cyclinder.

(3) Glassware with 125 ml capacity, for sample extraction.

(4) Whatman #1 filter paper or equivalent.

(5) Filter funnel.

(6) 100 µl pipette with disposable tips.

(7) Multi-channel pipette.

(8) 500 ml plastic squeeze bottle.

(9) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.

(10) Timer.

(11) Blender and blender jars.

(12) Balance.

(13) Sample Grinder.

11.10 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.